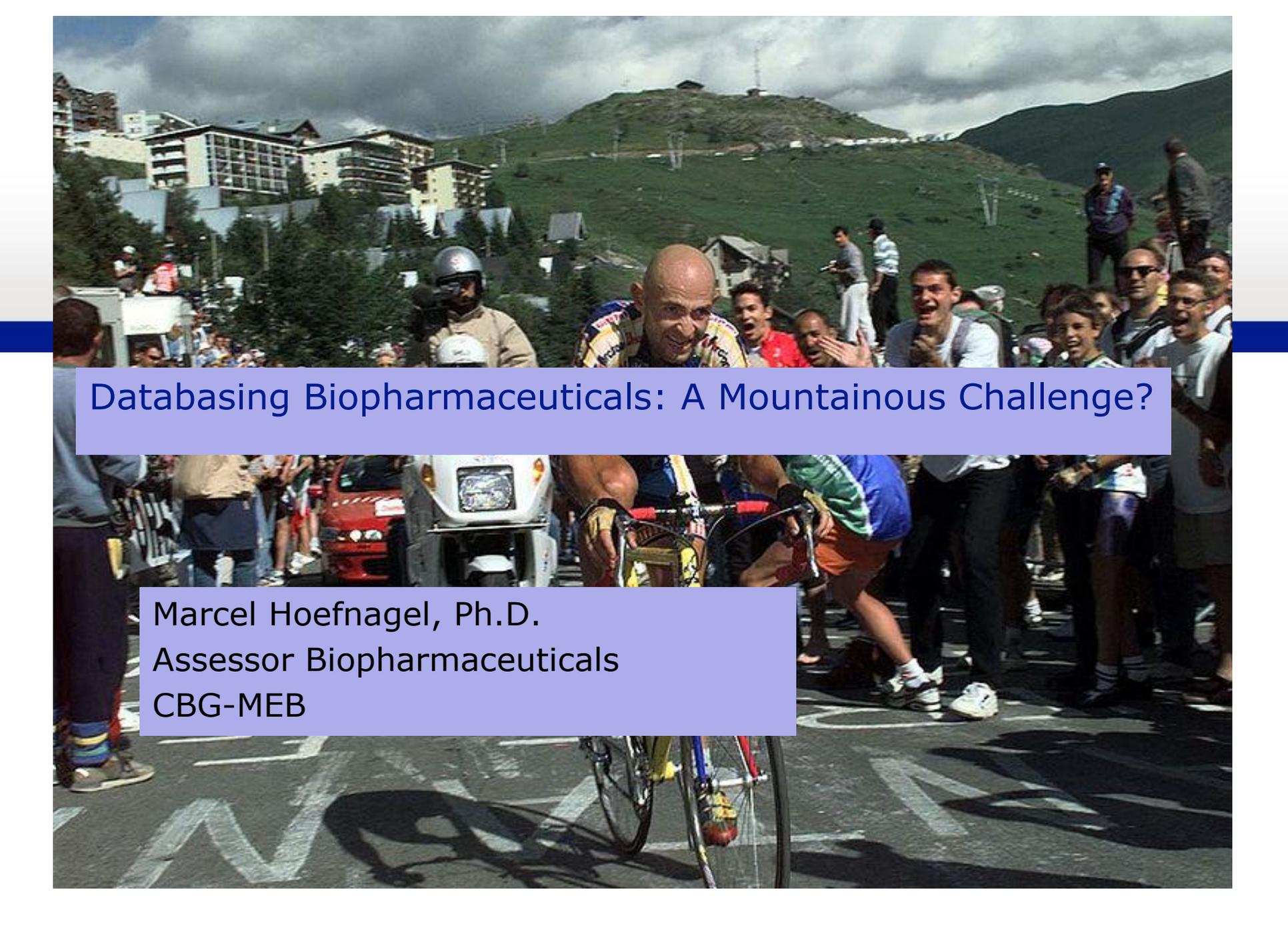


**C B G**  

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*M E B*

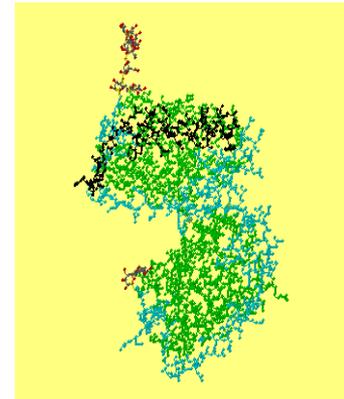
A photograph of a cyclist in a yellow and blue jersey racing through a mountain town. The cyclist is in the foreground, leaning forward on the handlebars. A crowd of people is gathered on the street, some cheering. In the background, there are multi-story buildings and a green hillside under a cloudy sky. A white van and a red car are visible behind the cyclist.

## Databasing Biopharmaceuticals: A Mountainous Challenge?

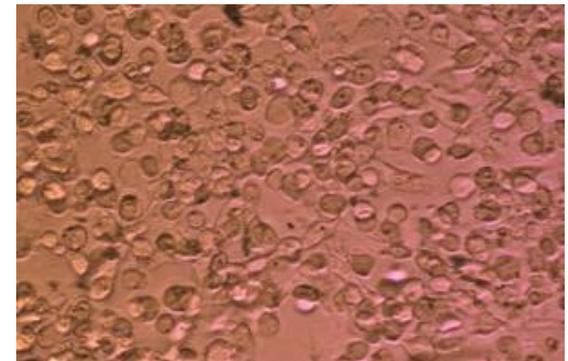
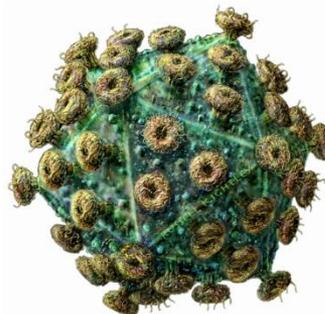
Marcel Hoefnagel, Ph.D.  
Assessor Biopharmaceuticals  
CBG-MEB

## “the following shall be considered as biological medicinal products:

- recombinant proteins,
- monoclonal antibodies,
- blood products,
- immunological medicinal products such as sera and vaccines, allergens,
- advanced technology products such as gene and cell therapy products.”
- Specific listed products ([www.hma.eu](http://www.hma.eu): gonadotrophins, heparins, non-rDNA proteins)



<b>ALLERGENIC EXTRACT</b>	
<b>STANDARDIZED CAT HAIR</b>	
<b>APT™ Acetone Precipitated</b>	
Strength: 10,000 BAU/mL	ITEM CODE mL
Dose/Route: See Package Insert.	Rx only.
Lot No.:	Standardized Cat Hair and Cat Pelt products are not interchangeable with each other or any other cat products including those labeled in AU/mL.
Exp. Date:	Store at 2° - 8° C.
Preservative: 50% (V/V) Glycerin	
Jubilant HollisterStier LLC Spokane, WA 99207 USA	U.S. Lic. No. 1272 202463 A



## Biologicals/biotechnologicals

- Legal text EC Directive 2003/63:  
Biological [active] substance is a substance that is produced by or extracted from a biological source and that needs for its characterisation and the determination of its quality a combination of physico-chemical-biological testing, together with the production process and its control ().



## Which aspects of Biologicals are relevant?

**Complex**

live virus/bacteria/cell  
inactivated virus/bacteria  
viral subunit/bacterial protein/bacterial-conjugate  
Plasma-derived proteins



**Simple**

Recombinant protein large/glycosylated  
Recombinant protein small/unconjugated  
Peptides

***Not complexity but heterogeneity makes life complex***

# Molecular size (bio)synthetic (poly)peptides

› Ga in menu naar Beeld > Model > Notitiemodel om deze titel te veranderen..

## Polypeptides / Proteins

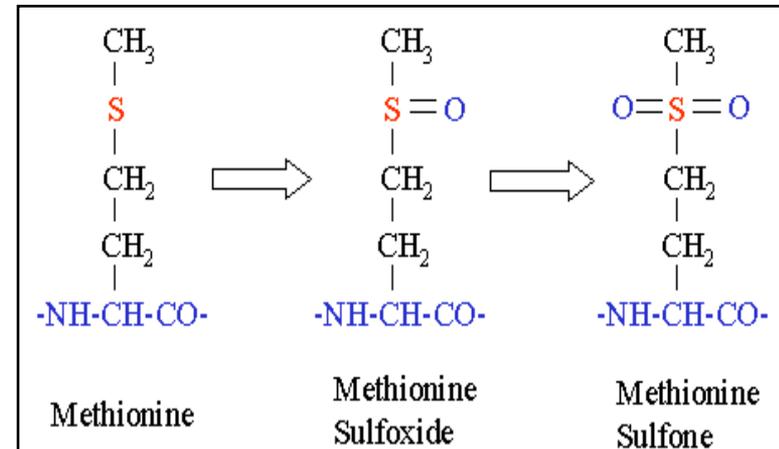
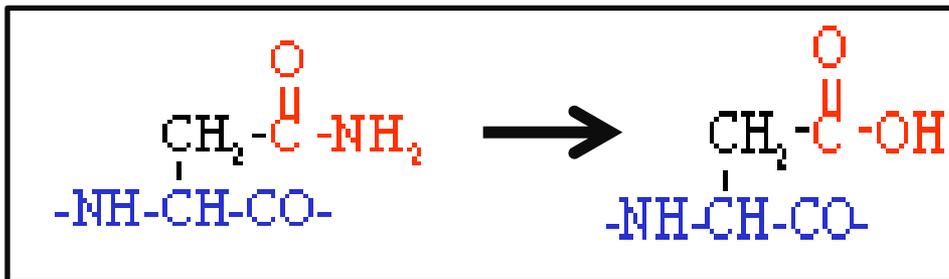
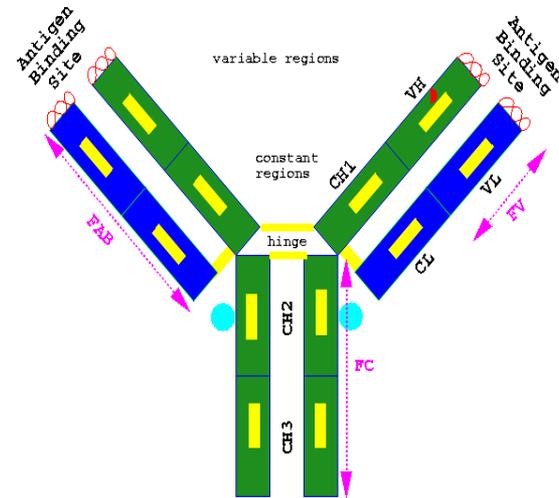
	# amino acids	mw
• Desmopressin	8 AA	1069
• Calcitonin	33 AA	3432
• Insulin	51 AA	5808
• Erythropoietin	65 AA	ca 30.600
• Interferon alpha-2b	165 AA	19.265
• Monoclonal IgG	1300 AA	ca 150.000
• Rec. FVIII	2332 AA	ca 280.000

Ga in menu naar Beeld > Model

CDLPQTHSLG SRRTLMLLAQ MRX<sub>1</sub>ISLFSCL KDRHDFGFPO  
 EEFGNQFQKA ETIPVLAEMI QQIFNLFSTK DSSAANDETL  
 LDRFYTELYQ QLNDLEACVI QGVGVTETPL MKEDSILAVR  
 KYFQRITLYL KKKYSPCAN EVVRAEDMRS FSLTSLNLES  
 LRSKE

## Recombinant proteins

- Which parameters?
  - AA sequence
  - Di-sulfide bridges
  - Glycosylation
  - Oxidation
  - De-amidation
- How relevant?



A professional cyclist in a yellow and blue jersey is racing down a mountain road. He is leaning forward on his yellow and blue road bike. A white motorcade vehicle is following him. A large crowd of spectators is lining the road, some cheering. In the background, there are buildings and a green hillside under a cloudy sky.

## Databasing EPO's: A Mountainous Challenge?

## ERYTHROPOIETIN CONCENTRATED SOLUTION

Erythropoietini solutio concentrata

```
APPRLICDSR VLERYLLEAK EAENITTGCA
EHCSLNENIT VPDTKVN FYA WKRMEVGGQA
VEVWQGLALL SEAVLRGQAL LVNSSQPWEP
LQLHVDKAVS GLRSLTLLR ALGAQKEAIS
PPDAASAAPL RTITADTFRK LFRVYSNFLR
GCLKLYTGEA CRTGD
```

$M_r$  approx. 30 600

### DEFINITION

Erythropoietin concentrated solution is a solution containing a family of closely-related glycoproteins which are indistinguishable from the naturally occurring human erythropoietin (urinary erythropoietin) in terms of amino acid sequence (165 amino acids) and average glycosylation pattern, at a concentration of 0.5-10 mg/mL. It may also contain buffer salts and other excipients. It has a potency of not less than 100 000 IU/mg of active substance determined using the conditions described under Assay and in the test for protein.

Ga in menu naar Beeld > Model > Notitiemodel om deze titel te veranderen..

# Glycosylation sites

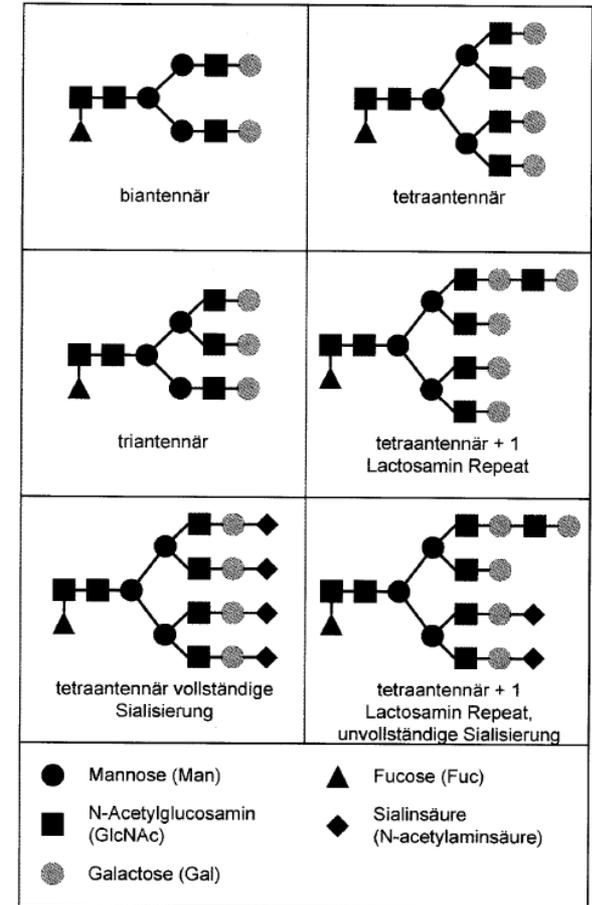
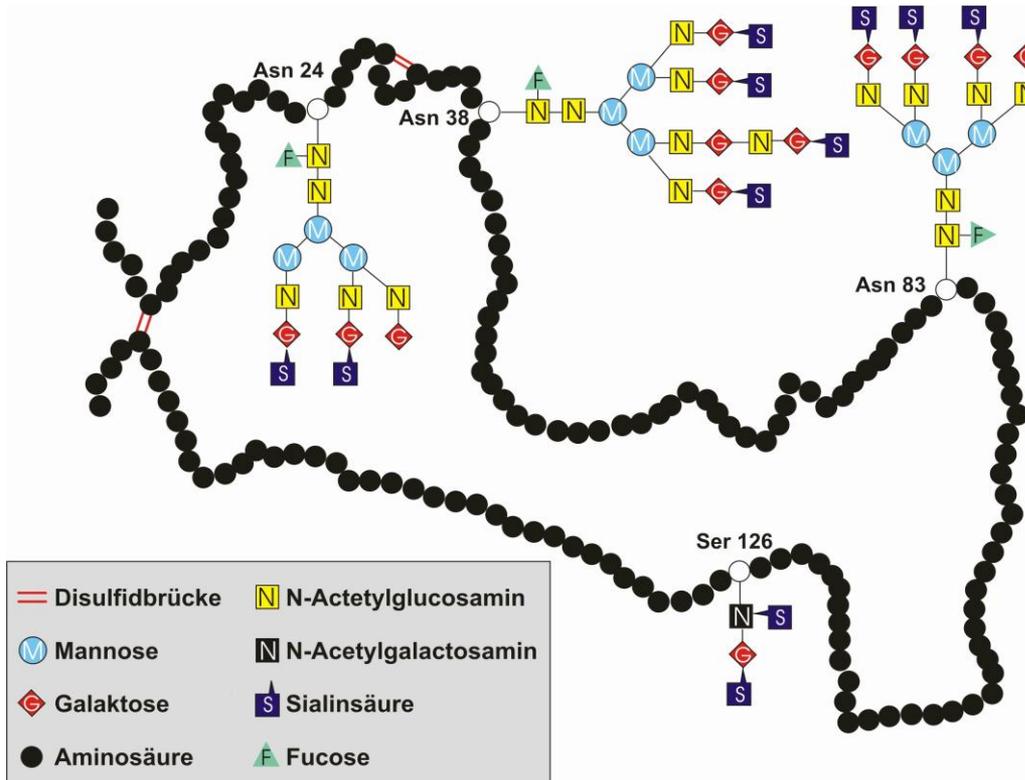


Abb. 2: Glykosylierungsvarianten des Erythropoietins.

► Ga in menu naar Beeld > Model > Notitiemodel om deze titel te veranderen..

## Isoforms in one batch

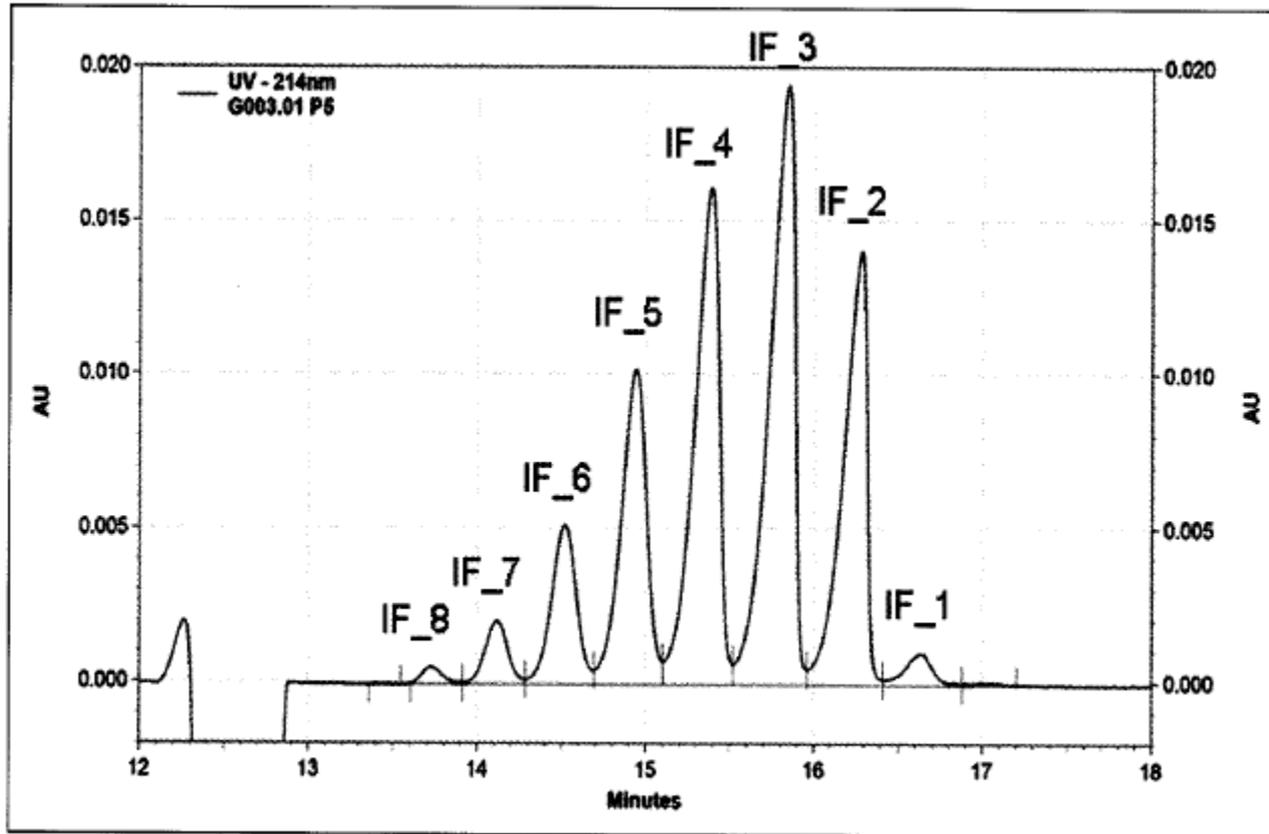


Abb. 3: Kapillarelektrophoretische Auftrennung des Erythropoietins.

EHC SLNENIT	VPDTK VNFYA	WKRMEV GQQA
VEVWQGLALL	SEAVLRGQAL	LVNSSQPWEP
LQLHV DKA VS	GLRSLT TLLR	ALGAQKEAIS
PPDAASAAPL	RTITAD TFRK	LFRVYSNFLR
GK LKLYTGEA	CRTGD	



$M_r$  approx. 30 600

## DEFINITION

Erythropoietin concentrated solution is a solution containing a family of closely-related glycoproteins which are indistinguishable from the naturally occurring human erythropoietin (urinary erythropoietin) in terms of amino acid sequence (165 amino acids) and average glycosylation pattern, at a concentration of 0.5-10 mg/mL. It may also contain buffer salts and other excipients. It has a potency of not less than 100 000 IU/mg of active substance determined using the conditions described under Assay and in the test for protein.

## Monograph on isoforms

Isoform	Content (per cent)
1	0 - 15
2	0 - 15
3	1 - 20
4	10 - 35
5	15 - 40
6	10 - 35
7	5 - 25
8	0 - 15

## Glycosylation is clinically relevant

- PK: without sialation very short serum half life
- Some isoforms less active than others

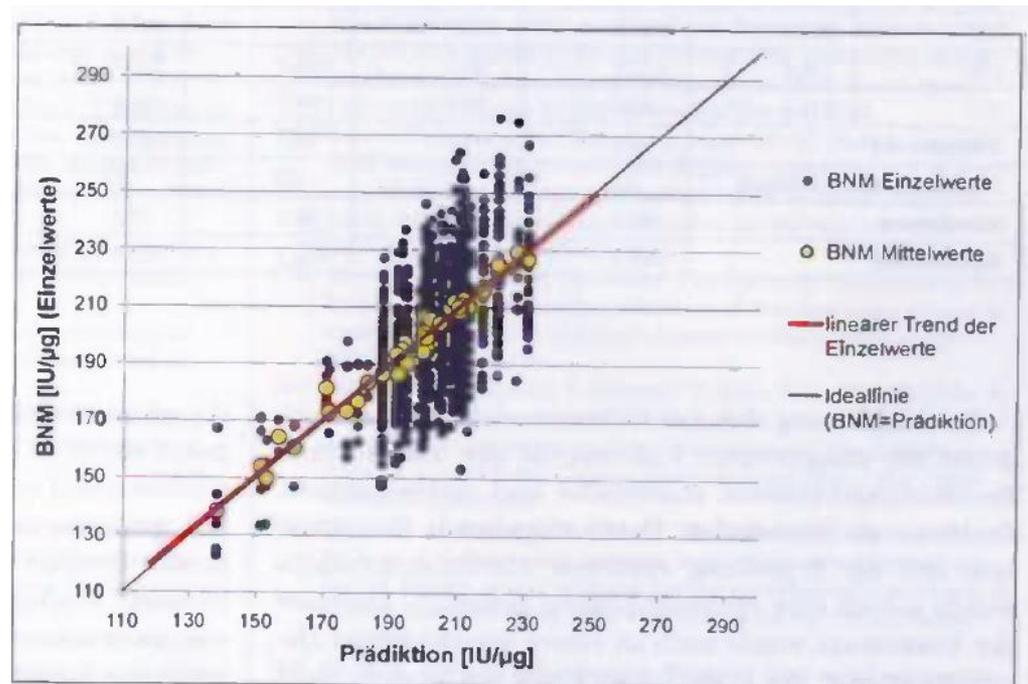


Abb. 14: Prädiktion der Trainingschargen.

## Isoforms in 40 batches (same manufacturer)

■ **Tabelle 2**

Zusammenfassung der Isoformenverteilung

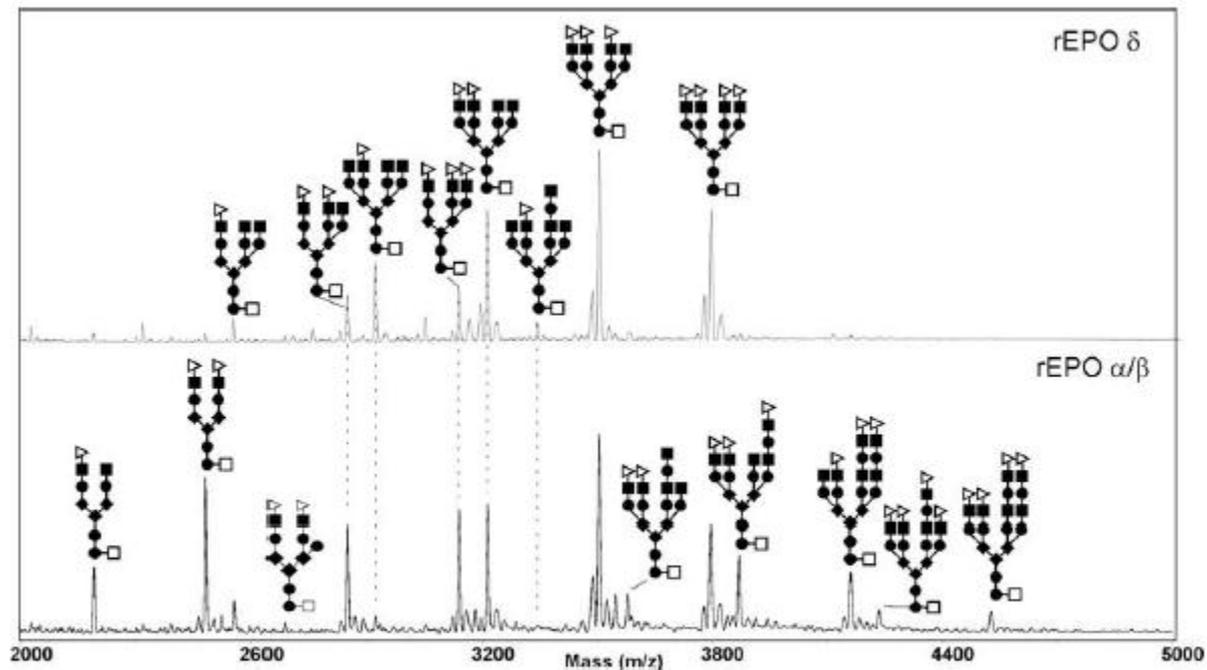
■ **Tabelle 1**

Biologische Aktivität der eingesetzten Wirkstoffchargen Zusammenfassung der Ergebnisse.

Auswertung der 40 „Trainingschargen“	Gesamt	Mittelwert	Minimum	Maximum
Biologische Aktivität	-	196 IU/mg	138 IU/mg	229 IU/mg
Bioassays pro Charge	1 057	26	3	71

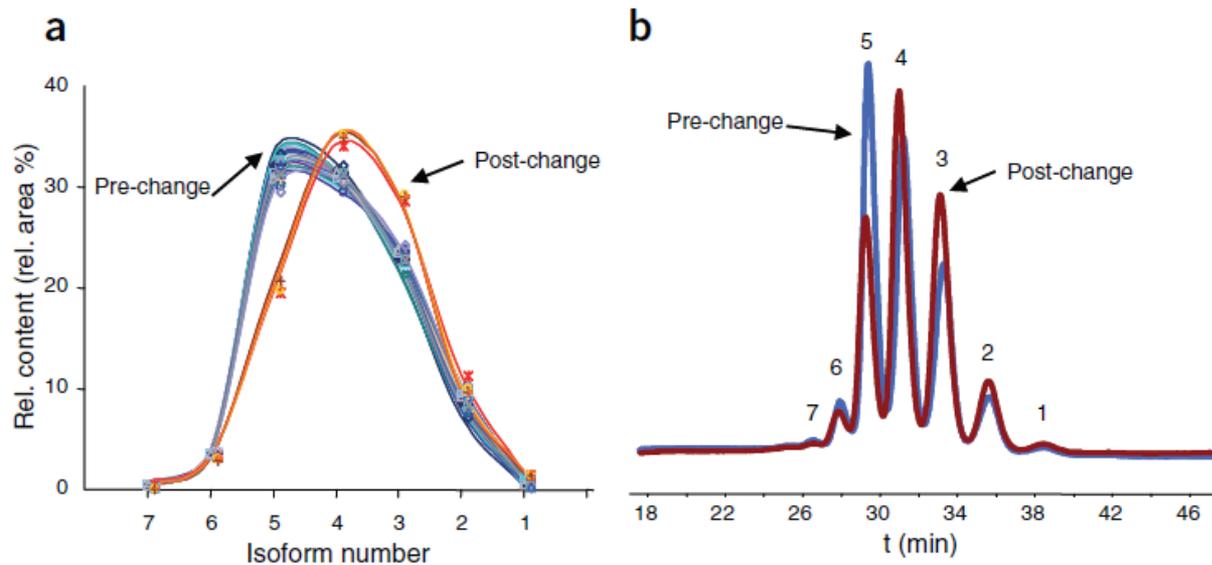
<b>IF_3</b>	26,48 %	14,93 %	31,48 %	10-35 %
<b>IF_4</b>	24,89 %	22,17 %	27,41 %	15-40 %
<b>IF_5</b>	15,25 %	9,12 %	22,93 %	10-35 %
<b>IF_6</b>	9,06 %	4,01 %	18,33 %	1-20 %
<b>IF_7</b>	4,63 %	2,04 %	11,13 %	0-15 %
<b>IF_8</b>	1,60 %	0,29 %	4,00 %	0-15 %
<b>IF_9</b>	0,16 %	0,00 %	0,47 %	-

## Glycosylation in EPOs with different INN



**Fig. 6.** Negative ion mode MALDI mass spectra of 2-AB-labeled glycans from rEPO  $\delta$  (top) and rEPO  $\alpha/\beta$  (bottom). The depicted structures indicate all possible isomers.  $\square$ , fucose;  $\bullet$ , N-acetylglucosamine;  $\blacklozenge$ , mannose;  $\blacksquare$ , galactose;  $\blacktriangle$ , sialic acid. The structural assignments for rEPO  $\alpha/\beta$  are based on NMR data. The structural assignments for rEPO  $\delta$  have been done by analogy to those of rEPO  $\alpha/\beta$  and are indicative only.

## Manufacturing process modifications



**Figure 1** Comparison of the pre- and post-change Aranesp batches measured by capillary zone electrophoresis. (a) Relative content of the individual isoforms of the pre-change ( $n = 18$ ) and the post-change ( $n = 4$ ) batches. (b) Representative electropherograms; peaks are labeled with the isoform number.

## Glycoproteins

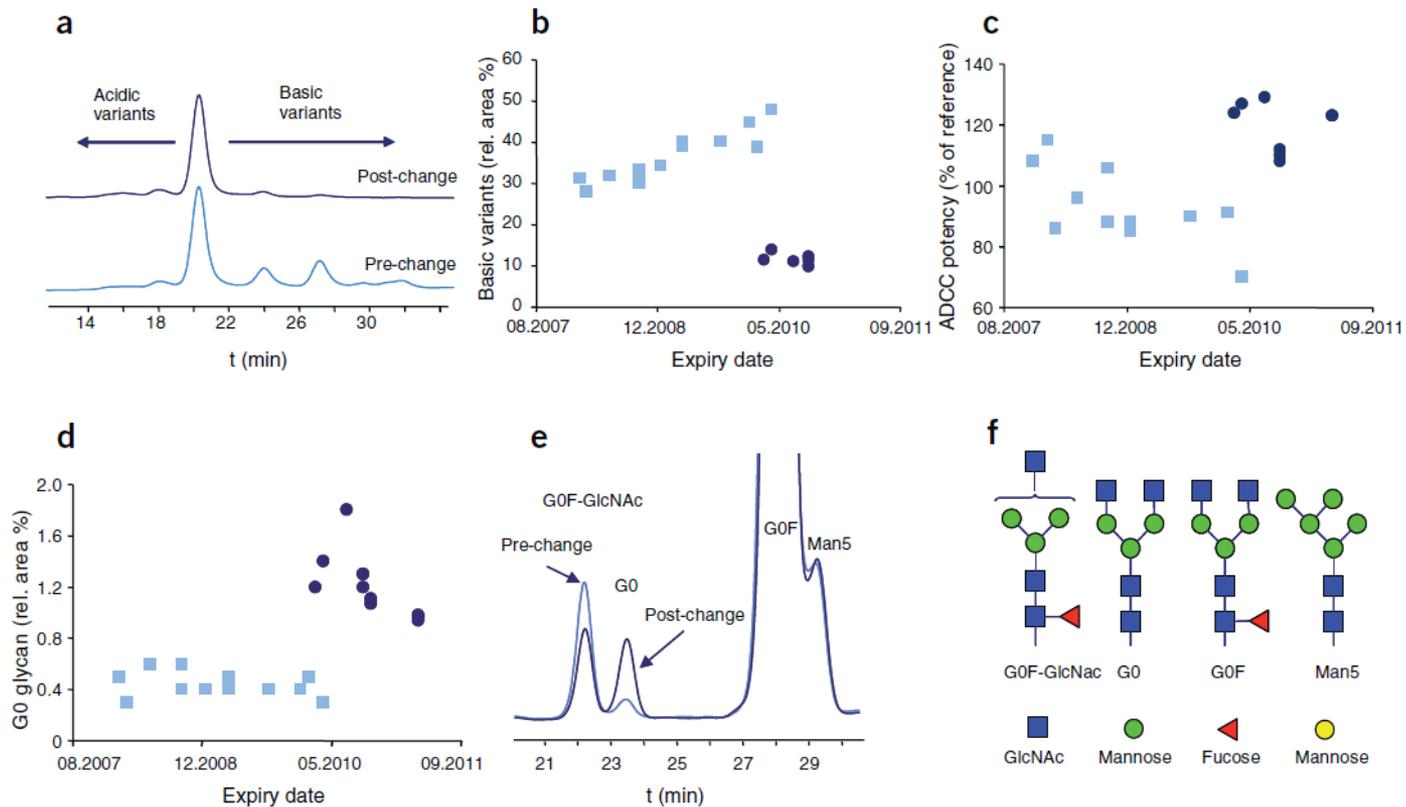
Which isoforms have to be documented?

- Everything >1%, >5%?
- Specific Glycogroups (e.g. EPO contains up to 1% NGNA, non-human glycosylation)?
- Product Ranges of the batches

Which differences relevant?

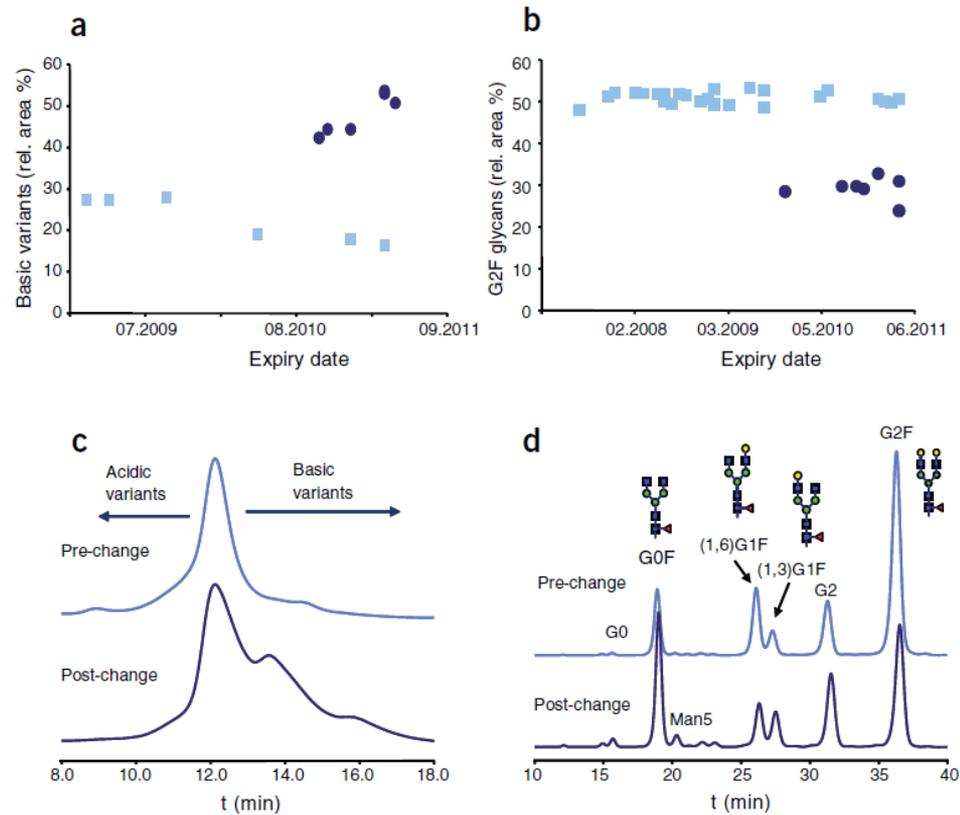
- Biosimilar EPO not necessarily same glycosylation

## Manufacturing process modifications (2)



**Figure 2** Comparison of the different pre- and post-change batches of Rituxan/Mabthera. (a) Exemplary CEX chromatograms. (b) Amount of basic variants of the pre-change ( $n = 12$ ) and post-change ( $n = 6$ ) batches as measured by CEX. (c) ADCC potency of the pre-change ( $n = 11$ ) and post-change ( $n = 8$ ) batches. (d) Relative amount of the G0 glycan of the pre-change ( $n = 13$ ) and post-change ( $n = 11$ ) batches. (e) Exemplary glycan mapping chromatograms. (f) Glycan legend.

## Manufacturing process modifications (3)



**Figure 3** Comparison of the different pre- and post-change batches of Enbrel. (a) Relative amounts of basic variants of the pre-change ( $n = 6$ ) and the post-change ( $n = 6$ ) batches as measured by CEX. (b) Relative amount of the G2F glycan of the pre-change ( $n = 25$ ) and the post-change ( $n = 9$ ) batches. (c) Exemplary CEX chromatograms. (d) Exemplary glycan mapping chromatograms.

## Differences after process modifications

- Are any of these changes clinically relevant?
- Are there other examples?
- Similar for various other quality parameters (e.g. impurities)
- A database containing also this information is very valuable.
- Similar compounds should be found (e.g. same protein missing one terminal AA should still be recognised).

## Discussion

- All biologicals in database
- Complexity determines the type of parameters
- Heterogeneity is the real challenge
- Database will advance knowledge
- Database will allow comparison

**Complex**



**Simple**

***Not complexity but heterogeneity  
makes life complex***

**C B G**  

---

*M E B*